HIV Detection from Enzo Diagnostics

The Enzo Microplate Assay*for HIV DNA

for direct detection and quantitation of HIV virus

The ***Enzo Microplate Assay for HIV DNA*** is an easy-to-use, rapid and nonradioactive kit for detecting HIV DNA. It has wide application to research in epidemiology, diagnosis and treatment. The straightforward assay procedure is carried out in a microtiter plate or microwell strips. The generation of a color, which can be easily measured, indicates a positive reaction.

The ***Enzo Microplate Assay*** provides materials for the colorimetric detection of HIV proviral DNA or viral RNA which has been reverse transcribed. When used in combination with gene amplification techniques, this test provides several advantages over current procedures. Unlike Southern blots and other hybridization techniques, no radioactivity, no electrophoresis and no film are used. No specialized equipment, other than a standard microplate reader, which is present in most medical research labs, is required. In fact, for plus/minus determinations the results can be read by eye. The assay format uses either a microtiter plate, where up to 96 assays (including controls) can be run, or well strips, where 8 assays per strip (including controls) can be run. Assay of the amplified DNA requires less than three hours. Thus, when combined with the amplification step, samples can be processed in a single day.

The ***Microplate Assay*** detects HIV proviral DNA with excellent sensitivity. The data presented in Figure 2 show that fewer than 10 HIV proviral sequences can be detected. Furthermore, when the assay is performed using a standard curve of HIV DNA copies, quantitative virus measurements can be done. Thus, the ***Microplate Assay*** is readily applicable to studies measuring the effect of drug treatments on virus concentration, virus concentration during the course of infection, virus concentration in animal model studies and numerous other studies where virus quantitation is a critical parameter.

Direct and quantitative HIV detection offers a distinct advantage over serological assays. The identification of provirus DNA can be made independent of the presence or absence of antibodies, which appear several months after initial infection. Thus, this technique offers researchers the potential for identifying proviral DNA in instances where HIV positivity can not be identified adequately by any of the current means available as, for example, in samples from HIV positive individuals who have not seroconverted.

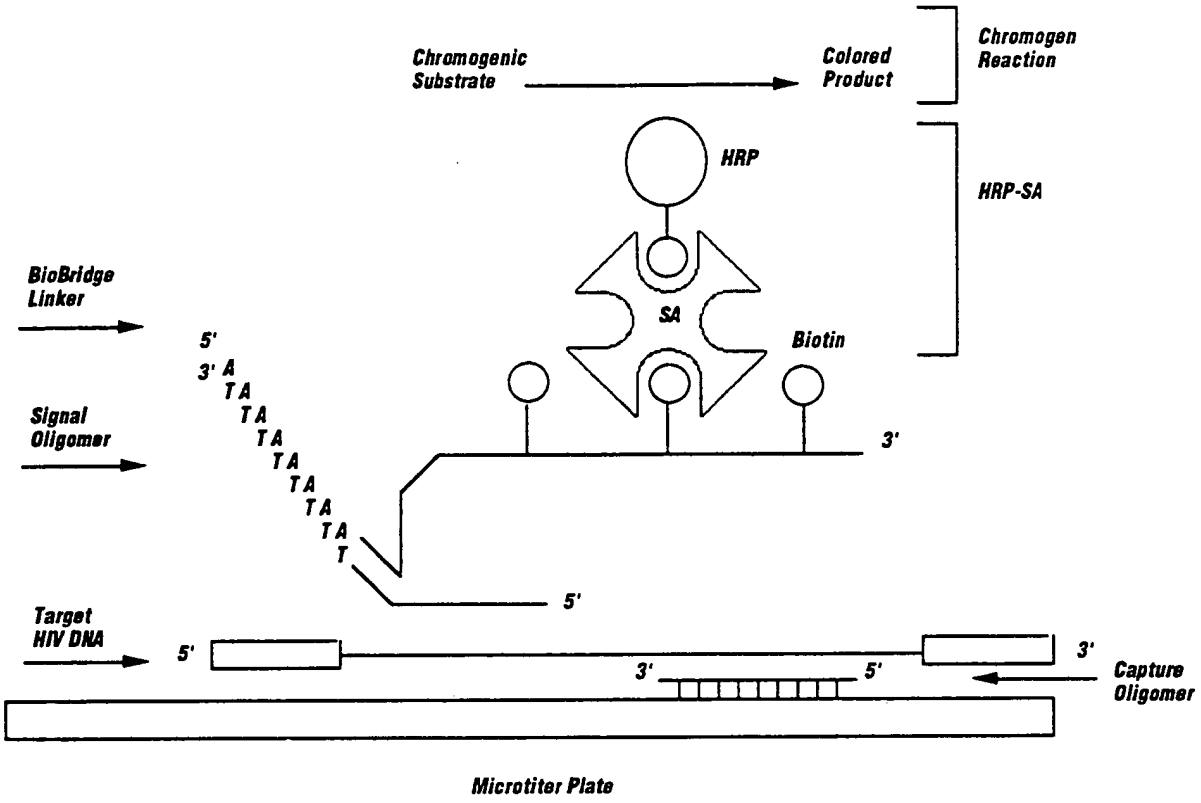
Reference:

Cook, A.F., Vuocolo, E. and Brakel, C.L. (1988) Synthesis and hybridization of a series of biotinylated oligonucleotides. Nuc. Acid Res. 16:4077-4095.

The Enzo Microplate Assay for HIV DNA
Catalog No. 46330

**U.S. Patent Nos. 4,711,955, 4994,373 and patents pending*
The Enzo Microplate Assay is for research use only

Figure 1.
Schematic Representation of the Enzo Microplate Assay for HIV DNA



PROCEDURE

The ***Enzo HIV Microplate Assay Kit*** provides materials for the colorimetric detection of nucleic acid containing the gag region. The method is based on a two probe hybridization procedure performed in a 96-well microtiter plate or a microwell strip. HIV DNA can be assayed directly if there is sufficient target DNA present, or it can be assayed indirectly in procedures employing target amplification. The nonradioactive procedure involves hybridization of target nucleic acid to a well-bound capture probe followed by binding of a biotinylated signaling probe to the captured target. Immobilized target DNA is then visualized by reaction with a biotin-binding signal generation complex of streptavidin (SA) and horseradish peroxidase (HRP). A positive reaction is indicated by generation of color which can be measured by a microplate reader commonly used in the laboratory. Using this format, 10^7 to 10^8 copies of target sequences can be detected.

DENATURE SAMPLE
Incubate sample with buffer for 15 minutes to denature the target nucleic acid sequences.

HYBRIDIZE TO WELL-BOUND CAPTURE PROBE
After rinsing the wells, add sample to each well and incubate for 90-120 minutes.

REACTION WITH SIGNAL PROBE
After the sample DNA is hybridized to the capture probe, signal probe is added and incubated for 15 minutes.

ADDITION OF LINKER
After rinsing the wells, add the linker (which supplies the biotin) to each well and incubate for 10 minutes.

DETECTION
Add the streptavidin-horseradish peroxidase complex, and then add the chromogen/substrate to generate color.

- *A positive result appears as a blue color which turns to yellow upon addition of the stop solution.*
- *Results may be quantified by reading OD at 450.*

Abstract

Presented at US-Canadian Academy of Pathology Meeting
Boston, Massachusetts, March 1990

A NON-ISOTOPIC HYBRID CAPTURE ASSAY FOR HIV NUCLEIC ACID SEQUENCES.

L.S. Lee, H. Payne, C.-Y. Ou, G. Schochetman and W. Maltzman, Enzo Biochem, New York, NY and Centers for Disease Control, Atlanta, GA.

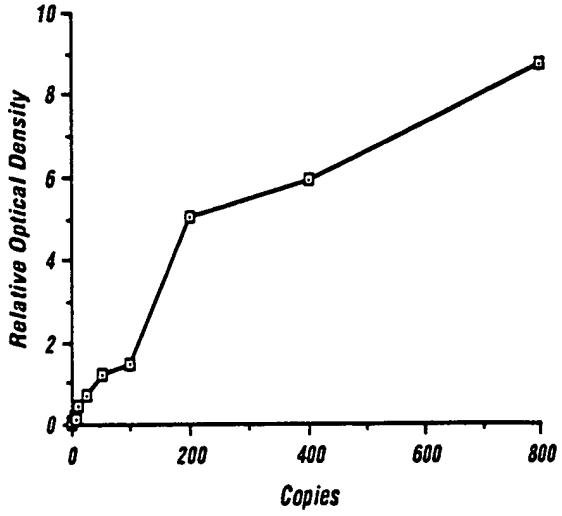
Oligonucleotide sequences were chosen from two relatively conserved regions of the human immunodeficiency virus type 1 (HIV-1) genome. These were then employed in a microtiter plate assay which involves the capture of target nucleic acid by virtue of its complementarity to an immobilized capture oligonucleotide. Hybridization is detected by incubation with a second specific signal oligonucleotide, using an enzyme-dependent amplification system to yield a colorimetric readout. This strategy has been applied to detection of HIV DNA and RNA sequences in both model systems and clinical specimens.

In experiments where enzymatically amplified HIV DNA, from either the gag or env regions, was the target, we were able to detect HIV DNA in all (36/36) samples from seropositive individuals. These results were in agreement with a radioactive "gel assay" performed in parallel on the same samples. The results of reconstruction experiments in which known amounts of HIV DNA were assayed in our nonradioactive system suggest that application of this technology to the problem of detection of HIV in clinical samples might allow the identification of individuals who harbor low levels of HIV proviral or viral nucleic acid, *e.g.*, prior to seroconversion. To date, we have detected HIV DNA after amplification in 6/6 samples taken from individuals who were seronegative at the time of sampling, but who subsequently seroconverted.

Figure 2.

DETECTION OF HIV SEQUENCES IN AMPLIFIED SAMPLES OF CONTROL REACTIONS

Sample	*Copies*	*Relative Optical Density*
2393	800,000	93.45
2410	80,000	38.28
2398	8,000	15.38
2391	800	8.74
2390	400	5.91
2395	200	5.03
2400	100	1.44
2394	50	1.19
2403	25	0.682
2397	12.5	0.455
2399	6.25	0.104
2402	3.12	0.170
2392	1.56	0.038
2396	0.00	0.014
water	0.00	0.003



Samples represented 1μg of human DNA which was amplified for 35 rounds in the presence of the indicated number of copies of cloned HIV DNA. Relative optical density was read at the termination of the detection reaction and normalized to 2 μl of the undiluted amplification reaction product. In all cases OD's of greater than 1.00 were based upon assays of diluted samples that gave OD readings between 0.1 and 1.0.

To place an order or for technical assistance

Call:
800-221-7705
Toll Free in the U.S. and Canada.

516-496-8080
In New York State.
Between the hours of 8:30 AM - 5:30 PM Eastern Time.

Telex:
424683-ENZOBIO

Telefax:
516-469-0830

Write:
Enzo Diagnostics, Inc.
40 Oak Drive
Syosset, New York 11791

Ordering Information

- **Minimum Order** - $50.00
- **Terms** - Net 30 days
- **Telephone orders** - must be followed by written confirmation which must be marked *clearly as a confirmation.*
- **Shipping charges** - F.O.B. Syosset, New York
- **Claims** - shipping damages or shortages must be made within five (5) business days of receipt of order and accompanied by a written statement. Permission to return products will not be given unless authorization is received from Enzo Diagnostics. Products must be returned within five (5) days after receipt of order.
- **Price** - The price in effect at the time of shipment will determine the billing price.

Research use only

Unless otherwise specified, these products are for research use only and are not intended for diagnostic or therapeutic use.

Limited Warranty

These products are offered under a limited warranty. The products are guaranteed to meet all the appropriate specifications described on our data sheet at the time of shipment. ENZO Diagnostics' sole obligation is to replace the product to the extent of the purchase price. All claims must be made to ENZO Diagnostics within five (5) days upon receipt of order

Prices Subject to Change Without Notice.

Inquires for volume discounts are welcome.

Enzo Diagnostics, Inc.
40 Oak Drive, Syosset, New York 11791
800-221-7705, in NY 516-496-8080